

Role of the oligosaccharide attached to gamone 1 in the conjugation-inducing activity of the ciliate *Blepharisma japonicum*

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SUMMARY

The complementary mating types and II of the ciliate *Blepharisma japonicum* interact sexually via gamones. Gamone (produced by mating type) was the first glycoprotein discovered as a conjugation-inducing substance in ciliates. It was suggested that the oligosaccharide attached to gamone is an *N*-linked type without fucose modification. Recently, we investigated whether the oligosaccharide attached to gamone is indispensable for conjugation-inducing activity, using glycopeptidase-treated gamone. We found that gamone lacking the oligosaccharide showed much-reduced activity, suggesting that the oligosaccharide may be indispensable for inducing conjugation. In this study, we constructed a new expression system for gamone and expressed a recombinant gamone lacking oligosaccharide to obtain proof of involvement of the oligosaccharide in conjugation-inducing activity. Prepro- or mature-gamone was expressed from a pCold vector in *E. coli* strain JM109, by inducing with 1 mM IPTG for 24 hours at 15°C. The pCold vector is a cold-shock expression vector that can express Trigger Factor (TF) chaperone as a soluble tag. The products were separated into soluble and insoluble fractions, and the proteins were subjected to SDS-PAGE. Prepro- and mature-gamone 1 were found in both soluble and insoluble fractions, indicating that the pColdTF vector had facilitated efficient production of recombinant gamone in the soluble fraction. We purified the recombinant gamone with TALON Resin in batch procedure and removed the tags attached to the recombinant gamone with HRVC Protease. We investigated whether the recombinant gamone, which lacks the oligosaccharide, showed conjugation-inducing activity. The present study strongly supports our previous finding that the conjugation-inducing activity is associated with the oligosaccharide attached to gamone.